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Multi-electrode monitoring of guided excitation in patterned cardiomyocytes

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ABSTRACT

Guided excitation in patterned cardiomyocytes was analysed by extracellular recording of field potentials with a commercial device of multi-electrode array (MEA). In order to define fibronectin patterns on the MEA surface, microcontact printing was performed with a microscope-based alignment system. Cardiomyocytes were harvested from neonatal rats and then cultured on the patterned device. After 3 days, cell stripes were formed, allowing guided excitation along each of the cell stripes. Increasing the culture time from 3 to 5 days improved the beating repeatability without changing the quality of the electric conduction. More detailed analyses revealed that the excitation propagation along a cell stripe was sensitive to the cell coupling defects which could be modified by adding drugs in the culture medium during the early stage of the pattern formation. Our results suggest that extracellular recordings with patterned MEAs are reliable for quantitative analyses of guided excitation of cardiomyocytes.

1. Introduction

Monolayer aggregates of cardiomyocytes cultured on a substrate are often used to study rhythmic electrical and mechanical activities. Although such an approach does not provide realistic cardiac models, it allows developing important assays in developmental, regenerative and pharmacological biology without using animals [1–7]. Furthermore, they can also be cultured on a patterned surface and analysed using high sensitivity optical imaging techniques [8–11]. Accordingly, many investigations have been developed during the last decade, including drug sensitive gap-junctions [11–13], single cell dynamics [14,15], community effects of single cellular networks [6] as well as nanostructure regulation of construct functions [16] and three dimensional scaffolds for the formation of electrically excitable grafts [17].

In this work, we report on a method of investigation of patterned cardiomyocytes by using micro-contract printing (μCP) [18] and multi-electrode array (MEA) recording [5] techniques. Comparing to more conventional patterning techniques such as photolithography and lift-off [19], μCP can be simpler and more relevant to produce biochemical patterns on MEA surfaces without changing the MEA device configuration. Once the surface of MEA device is patterned, cardiomyocytes can be cultured to form excitable features. Then, the excitation propagation with or without external stimulation can be mapped by extracellular recording. Comparing to the intracellular recording with glass micropipettes, the extracellular recording with MEAs is much simpler and more reproducible, which also allows simultaneous measurements on multiple cell sites with high temporal and spatial resolution [20]. Previously, this technique has been used in investigations of cardiomyocytes such as contraction dynamics [21], scaffold induced anisotropy [21,22], stem cell differentiation [23–29], etc. The most of these investigations, however, were based on culture without patterning and much less effort has been devoted to the guided excitation along patterned cell features [30]. It is known that when dissociated myocytes are used in culture, new gap junctions can be formed between neighbouring cells. Drugs, patterning defects and some other physic-chemical perturbations should be able to affect the formation of these gap functions and consequently, the electric conduction of the cell layers. It is also known that in primary culture of the neonatal heart, both myocytes and non-myocytes (mainly fibroblasts) are present [31], which may significantly affect the tissue formation of cardiomyocytes. By adding growth inhibitors such as bromodeoxyuridine (BrdU), the proliferation of non-myocytes can be inhibited [32] so that the pattern formation and the electric conduction of cell layers can be improved. The purpose of this work is to study excitation propagation along well-defined cell stripes by extracellular recording with commercial MEA acquisition apparatus. Our results show that the electric propagation properties are both defect and drug dependent, thereby suggesting the usefulness of this method is pharmacological and tissue engineering studies.

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2. Methods and materials

Micro-contact printing has been done with a custom set-up to pattern fibronectin on the surface of a commercial MEA device (MSC, Germany), as shown in Fig.1. The stamp was mounted on a holder of diameter smaller than that of the culture chamber of MEA, which is placed on the *X-Y* stage of an inverted optical microscope (Olympus IX-71, Japan). The stamp holder itself is fixed to a rotation stage for angle adjustment and mounted on the top illumination lens holder of the microscope, allowing changing the distance between the stamp and the MEA device with *Z*-slider of the microscope.

The MEA device we used consists of 60 titanium-nitride electrodes of 30 μm diameter and 200 μm pitch size, patterned on a glass substrate in form of an 8-by-8 square lattice without four corners. These electrodes are insulated with a 0.5 μm thick layer of silicon nitride and individually connected to gold pads for outward wiring with a typical impedance of 200–400 kΩ at 1 kHz. To facilitate the cell culture experiments, the commercial MEA device was manufactured with a glass ring of 30 mm diameter.

The stamp was prepared by soft lithography using a mould produced by standard photolithography. For demonstration, 100 μm line-and-space grating was designed and the photolithography mask was obtained on a transparent film by a high resolution (3600 pixel $in.^{-2}$) laser printer. Then, the mask pattern was replicated into a thin layer of AZ-10XT photo-resist (MicroChem, Japan) spin-coated on a silicon wafer. After exposure and development, the resist pattern was exposed to trimethylchlorosilane (TMCS) vapour for 1 min. Afterward, a two component liquid pre-polymer PDMS mixing (Sylgard 184, Dow Corning, Japan) at a ratio of 1:10 was poured on the patterned silicon wafer with a thickness of about 2 mm and cured at 80 °C for 1 h. After peeling off, the PDMS stamp was cut into a disk of 6 mm in diameter and 3 mm in thickness with a biopsy punch (Kai medical Japan). Then, the stamp was washed with ethanol and dried under filtered air flow. In parallel, a PDMS column (stamp holder) of about 1 cm in diameter and 1.5 cm in height was prepared in a similar manner.

As ink solution, fibronectin (Sigma, Japan) was prepared with DI water at a concentration of 100 μg/mL. After deposition of the ink solution on the surface of the PDMS stamp, it was rinsed with water, blown dry with filtered air, attached on the PDMS stamp holder. Afterward, the PDMS stamp pattern was aligned with the MEA pattern (Fig. 1a) under microscope and the pattern transfer was done by bringing the stamp in conformal contact with the surface of the MEA device for more than 10 s (Fig. 1b). Finally, the patterned MEA device was vigorously rinsed by DI water in order to remove unattached proteins (Fig. 1c).

Primary cardiomyocytes cultures from ventricles of 1–3 day-old neonatal Wistar rats (SLC Inc., Japan) were prepared with a standard collagenase digestion protocol [33]. After harvesting, cells were suspended in Dulbecco-modified Eagle plating medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin streptomycin and 1% kanamycin and then pre-plated for 1 h in a tissue culture dish to reduce the number of fibroblasts. Unattached cells were collected and then plated on MEA samples at a density of 2–3 $\times$ 10^5 cells/cm^2. After 24 h incubation at 37 °C under humidified 5% CO_2 conditions, unsettled cells were washed away and the culture medium was replaced with minimum essential cultivation medium (MEM), supplemented with 10% calf serum, 1% penicillin streptomycin, and 1% kanamycin, with or without containing 10 μM BrdU (B-5002, Sigma, Japan). On the third day, the cells formed a confluent monolayer which exhibited spontaneous contractions.

Extracellular recordings of patterned cardiac cells were performed by using a PC-based data acquisition system with a 60 channel preamplifier of Multi-Channel Systems (MCS, Germany) and a 16-channel data analyser of AD Instruments (Bella Vista NSW, Australia). By changing the connection pins of the acquisition module, the whole MEA mapping area can be covered. Data were recorded at 10 kHz with 12-bit precision. To facilitate the measurements, the MEA device was taken away from the incubator and placed into the frame of the acquisition module of Multi-Channel Systems. During the observation, the temperature of the device was kept at 37 ± 0.1 °C with the help of the temperature controller manufactured by the same company (MSC, Germany). In addition to the electric measurements, optical images were taken to visualize the contraction of cardiomyocytes with a CCD camera mounted on the optical microscope (Q Imaging, Japan). Finally, the electric recordings were analysed off-line using software LabChart provided by AD Instruments (Bella Vista NSW, Australia) and compared to the corresponding optical images to have a more clear insight on the influence of pattern defects.

3. Results and discussion

By using our microscope-based μCP system, we were able to produce fibronectin patterns on the surface of commercial MEA devices with an alignment accuracy of a few micrometres (Fig. 1c). Cardiomyocytes could then be cultured on the MEA surface to form desired monolayer cell stripes. In the present study, the printing area was limited to 6 mm in diameter, which is much smaller than that of the culture area defined by the glass ring of the MEA device (30 mm in diameter), allowing the formation of large cell clusters outside the printing area. Such cell clusters were randomly distributed on the surface of non-patterned areas and showed

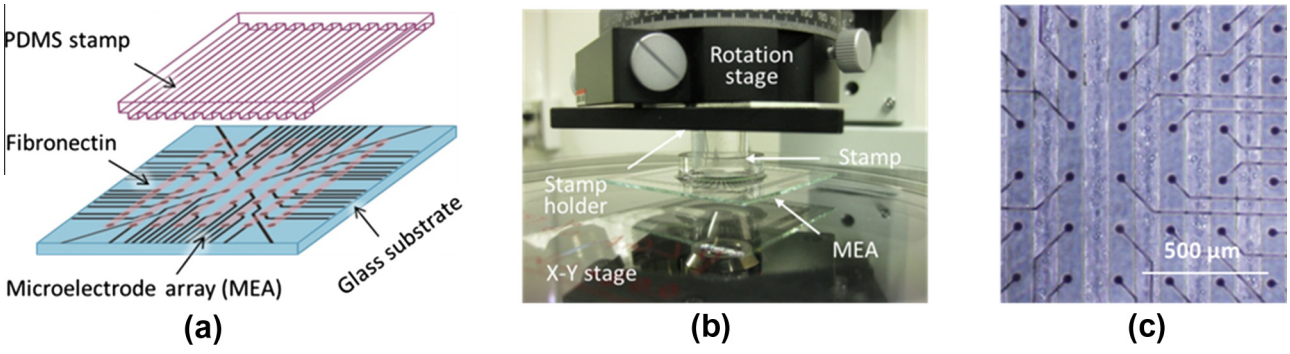

Fig. 1. Microcontact printing of fibronectin aligned on the surface of an array of MEA device: (a) Schematic of the printing configuration; (b) Photograph of the apparatus with a PDMS stamp mounted on a rotation stage which is fixed to the upside *Z*-slider of an inverted optical microscope. The MEA device is placed on the *X-Y* stage of the microscope; (c) Microphotograph of a patterned MEA surface.